REMARKS

Claims 1-5 and 9-15 have been allowed in the present application.

The specification has been amended to correct the listed authorship of the publication: Int. J. Artif. Organs, 1994, 17, 203-208; cited on page 5 of the specification. In the original text, the list of authors appears as Nakazawa et al. However, as evidenced by the enclosed copy of this manuscript, was cited on the Information Disclosure Statement filed on December 3, 2003, clearly shows that the authors should be Boccalatte et al.

No new matter has been added by the present amendment.

Applicants respectfully request that the amendment be entered.

Respectfully submitted,

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Amyloid bone disease and highly permeable synthetic membranes

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ABSTRACT: The effect of different highly permeable membranes on amyloid bone disease (ABD) was retrospectively evaluated in patients on renal replacement therapy (RRT) in our Unit with a dialytic age of more than 4 years. A group of 36 patients (age 60 ± 12 years) after a variable period (28 ± 29 months) on hemodialysis with cuprophane membrane (CU-HD), were moved to HDF with a reinfusate volume of 22 ± 1 1/session, for a period of 65 ± 26 months using the following membranes: AN69 1.6 m², PAN 1.8, PMMA 2.1, PS 1.3, polyamide (PA) 1.3 and 1.6. Bone x-rays of wrists, hips and shoulders were taken annually and the presence of ABD was evaluated according to generally accepted criteria. ABD occurred in 4 patients after a period of 73 ± 30 months on CU-HD only; it developed in 4/7 patients an AN69, in 4/6 on PAN, in 3/5 on PMMA, in 3/5 on PS; no patient of the 13 on PA developed ABD. Comparing patients on PA with those on other synthetic membranes, no significant difference was found in dialysis time (73 ± 19 vs 83 ± 28 months) as well as in age (59 ± 13 vs 61 ± 11 years) at ABD on set, when present. These data strongly encourage prospective studies enrolling more patients for a longer period of observation in order to evaluate possible differences on ABD development among various synthetic membranes. (Int J Artif Organs 1994; 17: 203-8)

KEY WORDS: Amyloid bone disease, Hemodiafiltration, Regular dialysis treatment, Highly permeable synthetic membranes

INTRODUCTION

Dialysis related amyloidosis (DRA) is a serious complication in patients on long-term dialysis treatment (1-3). After about 5 years of renal replacement therapy (RRT), the incidence of DRA increases, reaching 30 to 100% in patients treated for more than 15 years (2).

It has been shown that the amyloid fibrils consist of beta-2-microglobulin (B2-m), the major protein which accumulates in the plasma of chronically uremic patients and might play a role in tissue deposition of B2-m amyloid, resulting in carpal tunnel syndrome and bone disease (4, 5).

Amyloid bone disease (ABD) is characterized by the presence of multiple and symmetrical subchrondal cysts or articular erosions due to replacement of subchondral bone amyloid deposits (3, 6); these lesions have a tendency to increase in size and number (7).

In vitro studies suggested that the cuprophane membrane (CUM) might enhance B2-m generation (8, 9). Moreover, the prevalence of DRA has been reported to be higher in patients treated with CUM than in those dialyzed with acrylonitrile (AN69) M (10-14). On the other hand, the role of the dialysis technique is controversial, since no difference in B2-m plasma levels was observed between patients treated with CUM on hemodialysis (HD) and those treated with synthetic M on hemofiltration (HF) (14). However, studies on the kinetics of B2-m turnover suggest that HF could remove a larger amount of the B2-m produced compared to CU-HD (15).

Despite such conflicting data, it seems ascertained

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that long-term CU-HD results in DRA (10, 11). Therefore, the purpose of the present study was to evaluate possible differences in the various synthetic M on the onset and progression of ABD in patients treated in our Unit for over 4 years. This pathology was investigated by serial bone x-ray which is, at the moment, the best available technique for assessing DRA development (11).

PATIENTS AND METHODS

A group of 36 anuric patients (F 12, M 24, mean age 60 ± 12 years, body weight 65 ± 11 kg) was studied. None was suffering from amyloidosis causing diseases and all were at end-stage renal failure, due to chronic glomerulonephritis (CGN) in 18 patients, polycystic kidney disease (PKD) in 6, chronic interstitial nephritis (CIN) in 4, diabetic nephropathy (DN) in 3, nephrcangiosclerosis (NS) in 1, unknown etiology in 4. The patients had been on regular CU-HD treatment for a period of 28 ± 29 months and, then, moved to hemodiafiltration (HDF) for a period of 65 ± 26 months.

HDF was performed by employing the following M: AN69 1.6 m^2 (Filtral, Hospal) in 7 patients, PAN 1.8 (PAN 250, Asahi) in 6, PMMA 2.1 (Filtryzer, Hoechst) in 5, polysulphone (PS) 1.3 (Rapido, Bellco) in 5, polyamide (PA) 1.3 (Polyflux 130, Gambro) in 7 and PA 1.6 (Polyflux 160, Gambro) in 6. In a few patients at the beginning of the HDF period, a membrane other than the one subsequently used was employed for less than 12 months. In all cases QB was 400 ml/min; QD 500 ml/min, the dialysate composition was (in mEq/l) Na 138, K 3, Ca 3.5, Mg 0.7, acetate 38 for a period of 65 ± 35 months and, then, Na 141, K 2.5, Ca 3.5, Mg 0.7, Cl 104 ± 3, acetate 3, bicarbonate 40 ± 2 (Bicart System, Gambro) for a period of 29 ± 17 months; TMP was as high as 400 mmHg in order to obtain the highest possible UF, which allowed the removal of middle molecules, avoiding the risk of back-filtration and guaranteeing at the same time an optimal removal of small toxins (16); the session length was 217 ± 17 min; the postdilutional reinfusate was 22 ± 2 l/session with the following composition (in mEq/1): Na 138, K 2, Ca 3.5, Mg 1, Cl 109.5, acetate 35 for a period of 53 ± 26 months and, then, Na 139, K 2, Ca 3.5, Mg 1, Cl 101.5, acetate 4, bicarbonate 40 for a period of 15 ± 6 months (16).

No patient had been on aluminium (Al) containing phosphate binders in the last 10 years and dialysate Al was constantly well below the safe limit (< 3 mcg/l); thus, serum Al was constantly in the normal range in all patients.

Bone x-ray was taken for each patient every year from the beginning of RRT and evaluated by an expert radiologist at the end of the study period. In all 36 patients studied, technically adequate x-rays of hips, wrists and shoulders were available, allowing the ascertion of possible development of ABD. ABD was diagnosed according to previously reported criteria (11): cysts with a diameter of at least 5 mm in the wrists and 10 mm in shoulders and hips were taken into account; the cysts had to be located outside weight bearing areas of the joints and increase progressively in size. The entity of ABD was classified according to the following criteria: the site was indicated with Roman numbers I for wrists, II for shoulders, III for hips; the number of cysts was indicated with arabic numbers; the size of cysts was given a score: + (diameter 5-7.5 mm for wrists, 10-15 mm for hips and shoulders), ++ (7.5-10 for wrists, 15-20 mm for hips and shoulders), +++ (> 10 for wrists, > 20 mm for shoulders and hips).

The Mann-Whitney U test was used in order to compare different groups of patients.

TABLE I - PARAMETERS CONCERNING PATIENTS WHO DID (GROUP 1) AND DID NOT (GROUP 2) DEVELOP ABD AT THE END OF THE OBSERVATION PERIOD OR AT THE TIME OF ABD ONSET, WHEN PRESENT

Parameters		Group 1	Group 2
N. pts		14	22
Sex (F/M)		2/12	10/12
Age (years)		66 ± 10	58 ± 13
Total dialytic age (months)		82 ± 29	78 ± 24
Time on CU-HD (months)		38 ± 35	20 ± 21
Membranes	AN69 1.6 (n 7)	4	3
Employed	PAN 1.8 (n 6)	4	2
In HDF	PMMA 2.1 (n 5)	3	2
	PS 1.3 (n 5)	3	2
	PA 1.3 (n 7)	0	7
	PA 1.6 (n 6)	0	6

TABLE II - PARAMETERS OF PATIENTS OF GROUP 1 AT THE END OF THE OBSERVATION PERIOD, WITHIN THE BRACKETS THE TIME OF ABD APPEARANCE OR WORSENING AND THE EMPLOYED MEMBRANE. FOR THE EVALUATION OF BONE CYSTS SEE THE TEXT (METHODS SECTION)

Pts Sex	Age (yrs)	Dialytic age (months)		Bone cysts		
		on CU-HD	on HDF	site	number	size
G.B.	73	80 (62)	43 (20)	I	1	+
M			(AN69)	II	1	+
				III	1	+
O.C.	71	6	81 (67)	I	2	+
M			(PMMA)	II	1	+
				III	1	+
C.M.	72	2	159 (131)	II	1	+
F			(AN69)			
A.R.	66	8	91 (91)	II	1	+
M			(AN69)	III	3	+,++
P.C.	68	54	53 (12)	I	2	+
M			(PS)	III	1	+
R.M.	63	126 (115)	61 (12)	I	2	+
M			(PS)	III	2	+++
E.A.	68	48	50 (25)	I	1	+
M			(PMMA)	II	1	+
A.A.	68	77 (77)	50 (26)	III	2	+
M			(PMMA)			
C.P.	36	15	105 (69)	II	3	+
M			(PAN)	III	2	+,++
I.A.	68	29	78 (42)	I	1	+
F			PAN	III	2	+++
F.M.	78	22	105 (48)	II	2	++,+++
M			(AN69)			
M.I.	64	44 (38)	27 (12)	II	1	+
M			(PS)	III	1	+
A.B.	66	8	67 (31)	I	2	++
M			(PAN)	II	1	+
				III	1	++
B.C.	64	7	97 (36)	I	4	+,++,+++
M			(PAN)			

RESULTS

Of the 36 patients studied, 14 (39%) developed ABD (Group 1) and 22 (61%) resulted free from ABD (Group 2).

Group 1 (Tab. I and II). Two females and 12 males developed ABD after an average of 82 ± 29 months on RRT, including 38 ± 35 months on CU-HD, at a mean age of 66 ± 10 years. In 4 male patients of the above 14, ABD had already developed after a period of 73 ± 30 months on CU-HD and worsened progressively (both in size and number of cysts) after being moved to HDF (Tab. II).

HDF was performed with the following M.: AN69 in 4 patients, PAN in 4, PMMA in 3, and PS in 3. At the time of ABD onset, acetate dialysate had been employed for a period of 68 ± 31 and bicarbonate dialysate only in 3 patients for a period of 10 ± 9 months; acetate reinfusate had been used for a period of 43 ± 28 months and bicarbonate reinfusate was not utilized in any patient.

The mean intact sPTH (Nichols Institute, S. Juan, Capistrano, CA, USA) was 278 ± 223 pg/ml.

Group 2 (Tab. I). Of the 36 patients studied, 10 females and 12 males, mean age 58 ± 13 years, did not develop ABD after a period of 78 ± 24 months on RRT, comprehensive of 20 ± 21 months on CU-HD.

HDF was performed with AN69 in 3 patients, PAN

TABLE III - PARAMETERS CONCERNING GROUP A (PATIENTS ON HDF POLYAMIDE M.). AND GROUP B (PATIENTS ON HDF OTHER M.) At the end of the observation period or at the time of ABD appearance, when present. For the significance, see the text (results section)

Parameters	Group A (PA m.)	Group B (other m.)
N. pts	13	23
Sex (F/M)	7/6	5/18
Age (years)	59 ± 13	61 ± 11
Body weight (kg)	61 ± 7	67 ± 12
Total dialytic age (months)	73 ± 19	83 ± 28
Time on CU-HD (months)	20 ± 19	31 ± 39
Session length (min)	209 ± 10	221 ± 18
Reinfusate volume (l/session)	22 ± 2	21 ± 2
Time on acetate dialysate (months)	41 ± 26	69 ± 29
Time on acetate reinfusate (months)	37 ± 11	46 ± 25
ABD prevalence	0/13	14/23

in 2, PS in 2, PA 1.3 in 7, and PA 1.6 in 6. Acetate and bicarbonate dialysate was employed for a period of 50 ± 31 and 29 ± 18 months, respectively; acetate and bicarbonate reinfusate was used for a period of 44 ± 15 and 15 ± 5 months, respectively.

The mean intact sPTH was 249 ± 220 pg/ml and not significantly different compared to group 1.

On the basis of the above results, which pointed out a large difference of ABD prevalence between patients treated with PA and the other synthetic M (Tab. I), the 36 patients were then evaluated in 2 groups according to the synthetic M used (Tab. III).

Group A (Tab. III). This group included 7 females and 4 males treated with PA M; the primary nephropathy was CGN in 6 patients, PKD in 2, DN in 2, CIN in 1 and unknown in 1. The parameters concerning these patients at the end of the observation period are reported in Tab. III. In this group no patient developed ABD.

Group B (Tab. III). This group included 5 females and 18 males treated with synthetic M other than PA (7 with AN69, 6 with PAN, 5 with PMMA and 5 with PS); these membranes were coinsidered all together because of the similar prevalence of ABD in the patients treated. The primary nephropathy was CGN in 12 patients, PKD in 4, CIN in 3, DN in 1 and unknown in 2. In this group, ABD onset occurred in 4/7 patients on AN69, 4/6 on PAN, 3/5 on PMMA, 3/5 on PS. The parameters concerning these patients at the time of ABD onset are reported in Table III.

No significant difference between the 2 groups was found concerning primary nephropathy, time on RRT, previous time on CU-HD, age, body weight, reinfusate volume, time on acetate reinfusate. The time on acetate dialysate was significantly ($p < 0.01$) longer in Group B as well as the session length ($p < 0.05$). Sex prevalence could not be compared because of the unequal distribution in the 2 groups.

DISCUSSION

The pathogenesis of DRA is still uncertain and probably multifactorial. Since it is a serious complication of long-term RRT, over the last years many studies have been devoted to identifying the possible causes of such a disease (3). These studies, usually retrospective, are complicated by the fact that very few patients have received constant dialytic treatment during their RRT and by the difficulty of comparing different groups of patients because of changes in more than one variable of their mode of treatment such as dialytic membrane, dialytic technique, quality of water, dialysate and reinfusate composition (17).

Although the present study was not devoid of such methodological problems, it confirmed previous data (2) on prevalence and time of ABD onset. Moreover, it pointed out that factors other than time on RRT can play an important role for ABD development, since no significant difference in dialytic age was found between patients with and without ABD. Among the possible amyloidogenic factors, an important role has been advocated for the dialytic membrane (18-21) and, in agreement with this hypothesis, this study seems to suggest a possible lower prevalence of ABD in patients treated with PA M than those treated with the other synthetic membranes. In fact, no patient of the 13 on PA developed ABD, despite a mean time on RRT of about 6 years which is considered long enough for ABD onset (2).

Moreover, when the Group on PA was compared with that on the other synthetic membranes, in which 14 out of 23 patients developed ABD, no significant difference was found in the prevalence of primary

nephropathy, time on RRT, previous time on CU-HD, age, body weight, reinfusate volume and time on acetate reinfusate. Although age and time on RRT are considered the most important factors for ABD development (1-3, 11, 22), another variable such as time on acetate dialysate was found to be significantly different between the 2 groups and might play a role (23).

As far as the relationship between the dialytic membrane and DRA is concerned it was reported that long-term CU-HD results in this pathology (10, 11). In the present study, the previous time on CU-HD was found to be longer in patients treated with other synthetic membranes (Group B) than those treated with PA; this difference, although not significant, might have been contributing to the amyloidogenic process.

Previous papers attributed the relationship between dialytic membrane characteristics and DRA to two possible factors, that is the variable B2-m removal and the hypothetical B2-m generation stimulated by the membrane itself (11, 15, 18-21, 24-30). Regarding the former, convective and adsorptive clearances are implicated at different degrees for each membrane (15, 18-21) and a recent paper showed that PA M 1.3 in HF allowed a higher B2-m removal than PS 1.8 and AN69 1.6 m^2, although the amount of B2-m removed by PA 1.3 was lower than that produced weekly (31). Due to the retrospective nature of the present study, a longitudinal analysis of the B2-m plasma level was not available and this does not allow the exclusion of the possibility that a progressive reduction of these levels in the individual patient could delay DRA onset. Concerning the B2-m generation, this is indicated as one of the possible markers of membrane biocompatibility through different mechanisms (15, 18-21, 25-31).

In conclusion, if synthetic membranes employed in high convective processes delay DRA onset, as claimed by many authors, further studies on a larger prospective scale are needed in order to ascertain a possible advantage of the PA membrane on DRA development.

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